EDITORIAL

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Chick Embryo: A Preclinical Model for understanding ischemia reperfusion model

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Abstract

Ischemia-reperfusion (I/R)-related disorders, such as stroke, myocardial infarction, and other peripheral vascular disease, are among the most frequent causes of disease and death. Tissue injury or death may result from the initial ischemic insult, primarily determined by the magnitude and duration of the interruption in blood supply then by the next reperfusion-it induced damage. Various in vitro and in vivo models are currently available to review I/R mechanism within the brain and other tissues. Here, we developed an in ovo Hook model of I/R by occluding and releasing the proper vitelline artery of a chick embryo at 72 h of development. To validate the model and elucidate various underlying survival and death mechanisms, we employed imaging (Doppler blood flow imaging), biochemical, and blotting techniques and evaluated the necrobiosis mechanism: autophagy and inflammation caused by I/R. In conclusion, this model is beneficial in parallel with established in vitro and in vivo I/R models to know the mechanisms of I/R development and its treatment.

Ischemia was created in chick embryo by ligating the proper vitelline artery using sterile surgical suture. H Additionally, ranolazine, N-acetyl cysteine and trimetazidine were administered as an anti-ischemic drug to validate this model. Results from the present study depicted that blocking blood flow elevates HIF-1alpha, lipid peroxidation, peroxynitrite level in ischemic vessels while ranolazine administration partially attenuates ischemia driven HIF-1alpha expression. Endothelial cell incubated on ischemic blood vessels elucidated a higher level of HIF-1alpha expression with time while ranolazine treatment reduced HIF-1alpha in ischemic cells.

The incidence of ischemia-reperfusion (I/R) injury is high, and its pathogenesis involves complex, multifactorial, and it is also interrelated processes. I/R contributes to the pathophysiology of stroke, myocardial infarction, peripheral vascular insufficiency, and other thrombotic events. Prolonged ischemia results in detrimental cellular metabolic and ultrastructural changes. Thus, to attenuate or prevent irreversible cellular injury, restoring blood supply is important . Notably, reperfusion can augment the tissue injury compared thereupon produced by ischemia alone (Mathes et al., 2016; Tejada et al., 2016; Silachev et al., 2017). Thus, prompt revascularization and blood flow restoration, with minimal damage to the reperfused area, remaining the mainstay of all current therapeutic approaches for I/R (Linfante and Cipolla, 2016; Strand-Amundsen et al., 2018; Xiong et al., 2018; Yan et al., 2018). To mimic their a forementioned mechanism, suitable models closely resembling human pathology in clinical conditions are also needed, which will used contribute to our understanding of the mechanisms underlying I/R injury . Such models aid the understanding of I/R mechanisms and are also used in drug testing pipelines; ultimately translating to improved patient care.

In the last three decades, several critical factors which will act together to mediate the detrimental effect of I/R injury are identified. However, till date no treatment directed to I/R injury has shown to steer to an improvement in clinical outcomes. This is primarily due to the shortage of our complete understanding of the complexity of disease progression, and secondarily due to inappropriate research model selection. Currently, multiple species, including non-human primates, rodents, felines, and certain avian species, are utilized in I/R research. The disparity between the results obtained using these models and therefore the results of clinical trials, in humans, have led to the event of newer experimental model (Allen et al., 2005; Schmeer et al., 2008; Anvret et al., 2012; Xu et al., 2014; Gonzalez et al., 2015; Huang et al., 2016; Sommer, 2017; Yang et al., 2018). In this study, we used chick embryos as an alternate model to review the underlying mechanism of I/R injury.

Unborn embryos, like chick, zebrafish, and Xenopus, are extensively utilized in biomedical research. Chick embryos which are widely used due to their ready accessibility, ethical acceptability, relatively large size, cost effectiveness, and its fast growth (Seabra and Bhogal, 2010). Chick embryos have played an very important role in anatomical, embryological, developmental biology studies and that they are an efficient model for blood circulation research (Harvey, 1628; Lee et al., 2011; Smith et al., 2016). Furthermore, the vessel network of chick embryos are often a repository system for implanted human cells with none rejection (Wilson and Chambers, 2004; Deryugina and Quigley, 2009). Because the third day chick embryos possess a well-defined cardiovascular system , we selected a 72-h chick embryo as an appropriate model to review the I/R mechanism. The model used (hereafter mentioned because the Hook I/R model) within the present study and can effectively mimic all downstream pathway, e.g., oxidative and inflammatory pathways. Our model is straightforward , reproducible, and may be used for drug screening, and for routine I/R studies.

The experimental protocol for the use of chick embryos was submitted to the Era's Lucknow Medical College and Hospital's Institutional Animal Ethical Committee, which issued a written waiver stating that according to the Committee for the aim of Control and Supervision of Experiments on Animal (CPCSEA) no formal approval was necessary to perform these experiments. Though, Standard Operating Procedures were followed to attenuate any possible suffering by embryos.

To verify the efficacy of this model for conducting inflammatory research, we evaluated the expression of the NOD-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome pathway, and pro-inflammatory cytokines NF-kβ and IFNγ, involved in exaggerating inflammation. This study provided evidence for the activation of both the NLRP3 inflammasome as well as NF-kβ and IFNγ in response to I/R induced in the RVA.

Six hours of I/R increased the expression of NLRP3, cleaved caspase-1, ASC, and cleaved IL-1β by 1.4-, 0.9-, 0.83-, and 1.3-fold, respectively, and the changes in the levels of NF-kβ and IFNγ were 1.2-and 0.8-fold, respectively. The main advantage of generating a model is that it can be used to test the efficacy of drugs.

To verify that the Hook I/R model can be used for drug screening, we evaluated the protective effect of meldonium dihydrate (MD), trimetazidine (TMZ), MCC950, and N-acetyl cysteine (NAC). Several doses of these drugs were tested; the doses were chosen arbitrarily (with 10-fold increases in the concentration of the primary dose), or the doses selected were intermediate between the dose selected for cell culture and that used in animal models in other studies. The main advantage of generating a model is that it can be used to test the efficacy of drugs.

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